Herpetologica, 46(2), 1990, 169–175

SURFACE HABITAT ASSOCIATIONS OF THE OKLAHOMA SALAMANDER (*EURYCEA TYNERENSIS*)

RENN TUMLISON, GEORGE R. CLINE, AND PHILLIP ZWANK

Oklahoma Cooperative Fish and Wildlife Research Unit, Department of Zoology, Oklahoma State University, Stillwater, OK 74078, USA

ABSTRACT: Microhabitats identified by current speed, position in the stream, depth, and the substrate were evaluated in streams supporting populations of *Eurycea tynerensis*. Variables included substrate particle size, embeddedness, temperature, dissolved oxygen, current velocity, depth, and the relative abundance of isopods, ephemeropterans, plecopterans, amphipods, sculpins, and crayfish. Depth and velocity were negatively related to salamander occurrence and were the best indicators of suitable microhabitats. Substrate particle size, embeddedness, and presence of mayfly nymphs also were important predictors of salamander microhabitats. Salamanders were most commonly found in shallow (<10 mm), slowly moving (usually <10 cm/s) water with medium-sized rocks (65–256 mm diameter), moderate degrees of embeddedness (about 50%), and with high densities of aquatic invertebrates.

Key words: Caudata; Plethodontidae; Multivariate analysis; *Eurycea tynerensis*; Habitat; Ozark Mountains; Salamander

AN understanding of habitat selection is critical for conservation efforts for species with restricted distributions, and particularly for species Federally listed as endangered, threatened, or of special concern (such as the Oklahoma salamander). The Oklahoma salamander (*Eurycea tynerensis*) is a neotenic species restricted to the Springfield Plateau of Arkansas, Oklahoma, and Missouri and is typically found in "gravelly" (primarily chert) substrates (Moore and Hughes, 1939; Rudolph, 1978). Specifically, it inhabits interstices between stones and pebbles in coarse loose sand under cold swift shallow water of springs and small streams (Moore and Hughes, 1939). During drought, individuals live below the substrate surface (Dowling, 1956). Rudolph (1978) provided limited habitat descriptions of populations found near springs, but no evaluation of specific habitat requirements has been published.

Habitat selection can be inferred from variables that correlate strongly with the local abundance of an organism (Anderson and Shugart, 1974). For example, acidity affects habitat use by decreasing reproductive success in *Ambystoma maculatum* (Clark, 1986) and limits the distribution of *Plethodon cinereus* (Wyman and Hawksley-Lescault, 1987). Preference for pebble and cobble in stream bed habitat was demonstrated for *Desmognathus quadramaculatus* (Davic and Orr, 1987), and presence of fish predators affects habitat use in neotenic *Ambystoma gracile* (Taylor, 1983). We used statistical procedures to identify physicochemical and biotic factors that might be good predictors of microhabitat use by *E. tynerensis*.

METHODS

Habitat use was studied from May–July 1988. We selected survey sites in Oklahoma, Arkansas, and Missouri based on published records and accessibility as represented on 7.5 minute USGS quadrangle maps. When at least three *E. tynerensis* had been observed at a location, we scored all visually recognizable microhabitats according to substrate size, embeddedness, temperature, dissolved oxygen, current velocity, and depth. In addition, we recorded width of the wet channel in the vicinity of occupied microhabitats. We sampled 173 microhabitats at 46 locations. Microhabitats were identified by current speed, position in the stream, and nature of the substrate. Microhabitats represented the midstream and edges of runs, riffles, and pools; pools isolated from the main surface stream were included when present. Substrate particle size was coded according to a modified Wentworth scale (Bain et al., 1985). Embeddedness, which describes the degree to which substrate particles are covered by fine sediment, was coded using Platts et al. (1983). We evaluated embeddedness in terms of particle size that would clog interstitial spaces between larger substrate particles, and thereby limit escape by *E. tynerensis.* We only included particles <2 mm in diameter, and only scored embeddedness of the underside of substrate material because salamanders were not found above the substrate during daytime sampling. Current velocity was measured in centimeters per second (cm/s) with a pygmy current meter. All variables were recorded for microhabitats at which *E. tynerensis* was present and absent in each stream.

Substrate material was overturned in each microhabitat to evaluate the abundance of four aquatic invertebrate groups: mayflies (Ephemeroptera), aquatic sowbugs (Isopoda), stoneflies (Plecoptera), and sideswimmers (Amphipoda). Abundance was coded based on visual estimation by orders of magnitude: absent (0), present (1), common (2), or abundant (3). Common meant tens were seen per square meter, abundant meant hundreds were observed. Food habit analysis performed on stomachs of preserved specimens of *E. tynerensis* collected during this study demonstrated that these prey taxa were the important foods, accounting for 77.9% of the volume consumed (Tumlison et al., 1990). Occurrence (per square meter) of sculpins (*Cottus carolinae*) and crayfishes (primarily *Orconectes neglectus*), the dominant potential predators of *E. tynerensis,* was coded according to the scale: absent (0), 1–3 (1), or >3 (2).

Abundance estimates for *E. tynerensis* at each microhabitat sample were based on catch-effort data (numbers seen during 15 min of intensive searching in each available microhabitat). Abundance of salamanders was coded according to the same scale used for sculpins and crayfishes, which resulted in category sample sizes of 92, 35, and 46, respectively. The maximum number of salamanders located in one microhabitat was 20.

Means of variables in microhabitats with versus without *E. tynerensis* were compared using Wilcoxon two-sample tests for ordinal variables and analysis of variance (ANOVA) for continuous variables (Sokal and Rohlf, 1981). Relationships between habitat variables and coded salamander abundances were evaluated with Kruskal-Wallis tests. When these were significant, a stepwise testing procedure was used to determine where the differences occurred: Wilcoxon tests were used to compare abundances of 0-1, 1-2, and 0-2. If present, nonsignificant subsets could be found that indicate when the level of abundance is strongly influenced by the variable. For example, if the 0-1 comparison does not differ but 1-2 and 0-2 do differ, we infer that a high abundance value is strongly related to a particular state of the variable (indicating more optimal habitat).

Univariate analyses provide insights into underlying habitat selection, but results are confounded by dependence among variables (Anderson and Shugart, 1974). We used multivariate ordination techniques to eliminate effects of intercorrelations by extracting orthogonal variables that represented linear combinations of raw habitat variables. Multivariate ordination techniques have an additional advantage of deferring "noise" to smaller eigenvalues, thereby presenting structure expressing valid data relationships on primary vectors (Gauch, 1982*a*). We used reciprocal averaging (also called correspondence analysis) for ordination because, for most community data sets, it has been shown to be better than principal components analysis as an ordination technique (Gauch, 1982*b*). Discriminant analysis is commonly used to identify important variables, but the assumption of equal dispersion matrices is usually violated (Edge et al., 1987; Williams, 1983). This was true in our study, thus our canonical discriminant analysis is data-exploratory.

Use of relative abundance values for ordination of community data is desirable because biological processes responsible for abundances are of an exponential nature, causing a few dominant variables to control the results (Gauch, 1982*b*:212; Maarel, 1979). Quantitative data can be log-trans-

TABLE 1.—Variables used in analysis of *Eurycea tynerensis* habitats. Ranges and means are presented for samples in which salamanders were present (n = 81) and absent (n = 92). Observed significance levels (OSL) indicate the importance of the difference between means of each variable. Continuous variables indicated by * were analyzed by analysis of variance; ordinal variables were analyzed by Wilcoxon two-sample test.

	Range		Means		
Variable	Present	Absent	Present	Absent	OSL
Width (m)*	1–14	1–14	6.0	6.1	0.8540
Substrate	2–4	1–4	3.1	3.0	0.4861
Embeddedness	1–5	1–5	2.7	2.2	0.0005
Temperature*	12.0–25.2	12.6–23.8	17.0	17.0	0.8833
Oxygen (ppm)*	6.4–9.8	6.4–9.8	8.3	8.3	0.8377
Velocity (cm/s)*	1.0–25.0	0.0–75.0	4.1	18.0	0.0001
Depth (cm)*	1–7	2–38	3.1	11.7	0.0001
Isopoda	0–3	0–3	1.2	0.7	0.0021
Ephemeroptera	0–3	0–3	1.3	0.8	0.0004
Plecoptera	0–3	0–3	1.1	1.1	0.8633
Amphipoda	0–3	0–3	0.3	0.3	0.4926
Sculpins	0–2	0–3	0.3	0.8	0.0003
Crayfish	0–3	0–3	1.0	1.2	0.1411

formed, or data may be collected according to an abundance scale (particularly useful if sampling limitations restrict the collection of quantitative data). Rounding abundances to a scale with only a few values has little effect on resulting ordination scores, because algorithms for eigenvector ordinations involve an iterative process of averaging many numbers (Gauch, 1982*a*).

Width, temperature, and oxygen concentration were deleted from multivariate analyses due to low variation between microhabitats, and Amphipoda was deleted because it was restricted to only one of 11 drainages surveyed. All other variables were initially coded on an ordinal scale with the exceptions of velocity and depth. For multivariate analyses, velocities (cm/s) were coded as follows: 0–2.5 (1), 2.6–5.0 (2), 5.1–10.0 (3), >10.0 (4). Similarly, depths (cm) were coded: 0–4 (1), 5–10 (2), 11–15 (3), >15 (4). The intervals for these codes were chosen so salamanders would be represented in at least two of the categories.

RESULTS AND DISCUSSION

Wet width of the stream was not an important habitat variable (Table 1), and salamanders occurred in streams 1–14 m wide. Channel width reflects stream size, and to some degree the nature of microhabitats available, but the heterogeneous nature of some fairly large streams provided suitable microhabitats where *E. tynerensis* was found.

The coded mean substrate size occupied by salamanders was 3.1, which represents rocks in the 17–64 mm diameter class. Dundee (1958) noted this substrate size to be characteristic of the habitats of *E. tynerensis*. We found this variable to be our best initial indicator of streams occupied by *E. tynerensis*, yet substrate particle size was not significant in our microhabitat analysis. Sites producing no salamanders in our status survey were not included in the habitat analysis. Typically, such sites contained smaller gravel or bedrock. Thus, we feel the lack of discriminating power here is due to the microhabitat level of the analysis.

Habitat variables discriminating microhabitats with and without *E. tynerensis* were embeddedness, current velocity, and depth (Table 1). Mean embeddedness at sites with salamanders was 2.7, representing approximately 50% coverage of substrate particles by material <2 mm diameter. Pairwise comparisons (using α = 0.05) indicated that salamander densities were greatest where embeddedness was closest to 50%, and lesser with decreasing embeddedness. This degree of embeddedness presumably provided numerous interstitial spaces for salamander foraging and cover, while limiting the size of spaces and likely reducing risk of predation (Dundee, 1958). Streams with high degrees of embeddedness usually were devoid of salamanders and were not included in this analysis.

Salamanders were found primarily in slow currents averaging 4.1 cm/s. No salamanders were located where there was no current, or at current speeds greater than 25 cm/s (Table 1). Salamander abundance was negatively related to current velocity (Spearman correlation = -0.541, $P < 0.0001$). Salamanders were found at depths up to 7 cm ($\bar{x} = 3.1$ cm). Abundance was negatively related to depth (Spearman correlation = -0.691, $P < 0.0001$).

Availability of isopods and ephemeropterans was important in explaining occurrence of *E. tynerensis* (Table 1). Although salamanders were not found in several locations with abundant aquatic invertebrates, they were seldom present where these organisms were absent. The importance of these organisms may reflect their use as prey, or it may be correlated with habitat variables. Isopods and ephemeropterans tend to occur in unpolluted shallows, and isopods occur particularly in springs, spring-fed streams, and subterranean waters (Pennak, 1953). The presumed relationship of *E. tynerensis* with springs and Karst systems (Rudolph, 1978) is supported by the greater occurrence of isopods where salamander densities were highest (indicated by pairwise comparisons, $P < 0.05$). Presence of these potential prey is an important indicator of surface populations of *E. tynerensis*, although the relationship may actually reflect physicochemical properties of water in spring systems. Yet, Petranka et al. (1987) found that *Eurycea bislineata* responded positively to water conditioned with invertebrates. Thus, the relationship of salamanders and invertebrates may reflect predator response to chemical indications of prey. The relative rarity of *E. tynerensis* that we observed in Arkansas and Missouri corresponded to, but may not have been caused by, the low occurrence of invertebrates (unpublished data; this study).

Sculpins were found in deeper water than were salamanders, and the shallowness of the habitat occupied by salamanders generally excluded sculpins. The significance of sculpins, then, may mirror the importance of depth, as both factors were negatively related to salamander abundance. Laboratory experiments examining risk due to fish predators, including sculpins (Rudolph, 1978), indicated that *E. tynerensis* was the least susceptible of five species of larval salamanders, probably due to their thin bodies and ability to escape into interstices between rocks. However, Petranka et al. (1987) found that *E. bislineata* avoided water conditioned with a fish predator (*Lepomis cyanellus*). We found *E. tynerensis* in isolated pools and in shallow areas of the main stream beyond the reach of sculpins. The presence of sculpins may result in predator avoidance behavior and determine the substrate–surface distribution of *E. tynerensis*.

The ubiquitous distribution of crayfishes resulted in their statistical insignificance in accounting for the occurrence of *E. tynerensis*. Crayfishes occupied the interstitial spaces used by *E. tynerensis*, and tail loss, probably caused by crayfishes, was observed in 22% of the salamanders collected. Crayfishes may prey upon *E. tynerensis*, but we could not demonstrate that they affect salamander microdistribution.

Reciprocal averaging allows simultaneous display of ordinations of samples and variables along each principal axis (Gauch, 1982*a*; Greenacre and Vrba, 1984). The plot of scores (Fig. 1) indicates sculpins, crayfish, velocity, and depth to be negatively related and substrate, embeddedness, ephemeropterans, isopods, and plecopterans to be positively related to the first axis. Highest loadings were for depth, sculpins, isopods, and ephemeropterans. Sculpins were found in deeper water and aquatic invertebrates in shallower water, thus this axis represents a habitat gradient from faster, deeper to slower, more shallow waters. Accordingly, *E. tynerensis* tends to be absent from habitats with deeper and faster water and present in more moderate conditions. Variation accounted by the second axis did not provide infor-

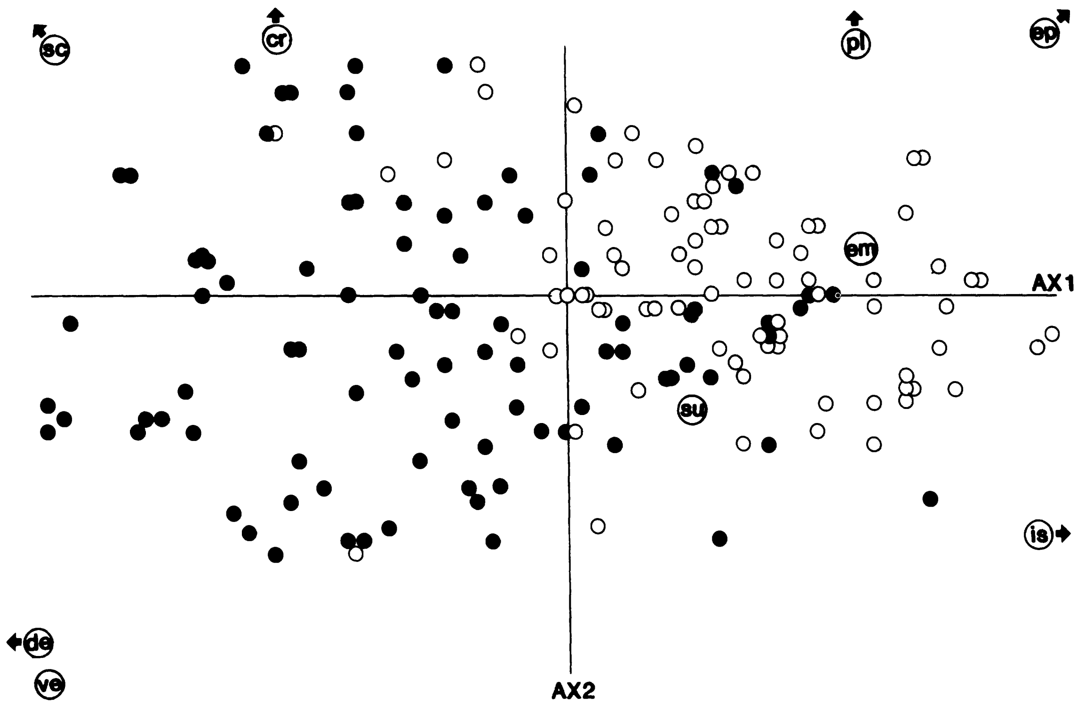

FIG. 1.—Reciprocal averaging analysis ordination of microhabitats occupied (open circles) and unoccupied (closed circles) by *E. tynerensis* in Oklahoma, Arkansas, and Missouri. The primary axis expresses a gradient from faster, deeper to slower, more shallow water. Habitat variables are also represented: sc = sculpins, cr = crayfish, de = depth, ve = velocity, su = substrate, em = embeddedness, pl = Plecoptera, ep = Ephemeroptera, and is = Isopoda. Coordinates for habitat variables were compressed for presentation; arrows indicate the direction of the true position.

mation useful in explaining microhabitat use.

We developed a canonical discriminant function using a stepwise procedure to select important variables that might allow limited presence–absence prediction capabilities. The function was

$$Y = 2.558 - 0.911(\text{Depth}) - 0.465(\text{Velocity}) + 0.264(\text{Ephemeroptera}).$$

Canonical variables evaluated at group means were 1.143 (present) and −0.994 (absent). The function correctly classified 86% of the samples used in its derivation. However, 95.0% of the presence sites but only 78.3% of the absence sites were correctly classified. Collection data accurately codes sites at which salamanders are present, but absence means only that no individuals were found during the sampling period. We interpret the canonical histogram (Fig. 2) to indicate that sites represented toward the left are inappropriate microhabitats. The zone of overlap near zero may represent marginal habitats that are occasionally occupied, and points to the right represent favorable microhabitat. The locations in this area labelled absent indicate sites that we feel would be good candidates for reinspection.

Caution should be used in making predictions based on our canonical function because (1) the assumption of equal dispersion matrices for the model was violated, (2) coding criteria may not be reproduced exactly by other investigators, and (3) seasonal or annual variations in biotic and physical characteristics of microhabitats may affect the relative importance of habitat variables.

Our analysis concerns only surface populations; subterranean habitat requirements are unknown. Dowling (1956) noted

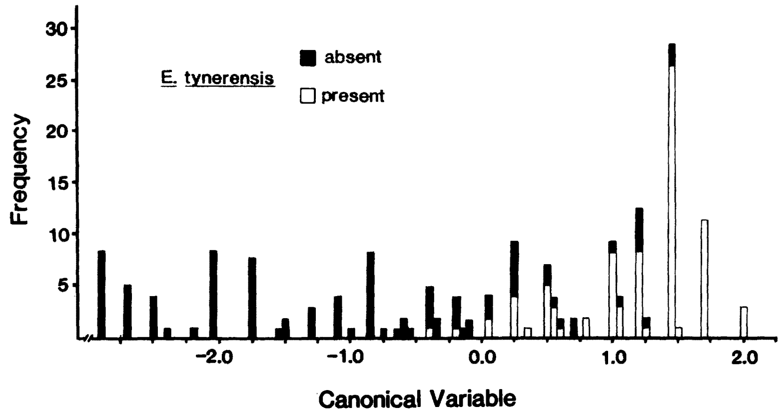

FIG. 2.—Histogram of canonical variable scores for each microhabitat sample. The mean value of negative sites is −0.994 and that of positive sites is 1.143. Overlap on the right indicates apparently suitable habitats that might produce salamanders with additional survey work.

that *E. tynerensis* moves below the substrate surface during drought. Our observations of two pools isolated from the main stream at Spring Creek, Mayes County, Oklahoma, indicated that salamanders may move beneath the surface to appropriate microhabitats. In the first instance, we found specimens 10 m from the surface stream in a pool with cool, slowly flowing water. The other pool was isolated in a mainstream gravel bar with deep, very swift current on either side. Those salamanders could have been isolated when water level increased, or they may have moved under the substrate and emerged at acceptable surface microhabitats. Further support for the latter possibility was obtained at Peavine Creek, Cherokee County, Oklahoma, where salamanders were found a few centimeters into the gravel substrate under shallow but swift water [similar to habitat described by Moore and Hughes (1939)]. Current in the substrate was much reduced, apparently making the microhabitat acceptable.

Our results suggest that multivariate techniques can provide additional insight to habitat utilization in salamanders. Discriminant analysis identifies habitat variables that are most reliable in distinguishing microhabitats in which a species is present versus absent. Simultaneous ordination of variables and sample plots (microhabitats in our case) through reciprocal averaging permits interpretation of relationships between variables and samples along the habitat gradient represented by each axis. More detailed analyses are possible for studies of greater depth (Gauch, 1982*b*; Greenacre and Vrba, 1984).

Our analyses are limited to streams in which *E. tynerensis* occurred in densities of $\geq 3/m^2$ in at least one microhabitat, thus the habitat gradient was short (i.e., we did not include streams with habitat characteristics that did not support populations of salamanders). For this reason, effects of variables such as acidity (which showed negligible variation within a stream site) could not be evaluated. Studies involving a longer habitat gradient and evaluated by reciprocal averaging often result in a bell-shaped response curve. This analytical problem can be overcome using detrended canonical correspondence analysis (Ter Braak, 1986, 1988).

Acknowledgments.—This project was funded by a grant from the U.S. Fish and Wildlife Service to Oklahoma State University. Research was conducted under the auspices of the Oklahoma Cooperative Fish and Wildlife Research Unit, Oklahoma Department of Wildlife Conservation, U.S. Fish and Wildlife Service, Oklahoma State University, and Wildlife Management Institute, cooperating. We thank A. A. Echelle, S. Fox, and G. Wilde for comments on the manuscript, and P. Bright for providing field accommodations.

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Accepted: 7 August 1989
Associate Editor: Raymond Semlitsch